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Effects of salt stress on volatile compounds, total phenolic content and antioxidant activities of *Salvia mirzayanii*

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ABSTRACT

Salvia mirzayanii is a medicinal and aromatic plant belonging to the Lamiaceae family, which is an endemic plant in Iran. In this study, the effects of different salt concentrations on total phenolic content, antioxidant activities and volatile components of the aerial parts of *S. mirzayanii* were studied. The results showed that total phenolic content increased with the increase in salt concentration. The increase was more pronounced under moderate salinity (3.8 mg GAE g^{-1} DW at 6.8 dS m^{-1} NaCl). Plants grown at 6.8 dS m^{-1} NaCl displayed the highest DPPH˙ scavenging activity with the lowest IC_{50} value (2.13 mg ml^{-1}) compared to the control. The volatile components were identified and analyzed by HS (headspace)-GC–MS using the Combi PAL System technique. The main components of control plants were α-terpinyl acetate, 1,8-cineole and bicyclogermacrene. The proportions of these main compounds were differently affected by salinity stress. The results showed that the synthesis of both total phenolic and some important volatile components was induced by moderate salinity.

1. Introduction

Medicinal and aromatic plants are increasingly used in several fields such as agroalimentary, perfumes, pharmaceutical industry and natural cosmetic products (Baatour et al., 2010). In the past few years, interest in the antioxidant properties of plant-derived foods and medicinal plants has increased, because plant antioxidants and essential oils are involved in preservation of human health (Giorgi et al., 2009).

It has been shown that the antioxidant activities of plant products are mainly due to phenolic compounds, such as flavonoids, phenolic acids, tannins and phenolic diterpenes (Lee et al., 2004). In this way, phenolic compounds play an important role in absorbing and neutralizing free radicals, quenching singlet oxygen, and decomposing peroxides (Ksouri et al., 2007). Phenolic compounds also exhibit a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, cardioprotective and vasodilatory effects (Balasundram et al., 2006). These compounds are the intermediates in the phenylpropanoid pathway and play important roles in flavonoid production and lignin biosynthesis (Zheng et al., 2001). The polyphenol synthesis and their accumulation are generally stimulated in response to biotic/abiotic stresses such as salinity (Ksouri et al., 2007). Indeed, polyphenolic compounds participate in plant protection against reactive oxygen species (ROS), which are inevitably produced when aerobic respiration or photosynthetic metabolisms are impaired by environmental stresses (Bettaieb et al., 2011). Thus, salt-stressed plants might represent potential sources of phenolic compounds for economical use.

In addition to phenolic compounds, the growth, yield and essential oil composition of the majority of medicinal and aromatic plants can be affected by environmental constraints such as salinity. Changes in both essential oil (EO) yield and their compositions have been reported to be influenced by environmental conditions (Gil et al., 2002). Some studies have shown a decrease in essential oil yields and changes in their compositions under salinity stress (Dow et al., 1981). On the other hand, there are reports in support of the stimulating effects of abiotic stresses on the synthesis of secondary metabolites in plants (Hendawy and Khalid, 2005).

Lamiaceae plants have been widely studied as natural antioxidant sources because of their high phenolic content and valuable essential oil component (Trouillas et al., 2003). Several genus of the Lamiaceae family such as *Salvia* species are used in folk medicine as antiseptics, astringents and spasmolytics. Many studies have indicated the antioxidant, antimicrobial and antiviral activities of some *Salvia* species (Yamini et al., 2007). *Salvia mirzayanii* Rech. F. & Esfand is one of the members of the Lamiaceae family which is a wild-growing flowering plant and belongs to the genus *Salvia* (Javidnia et al., 2002). It is a herbaceous biennial or perennial and an endemic plant growing in Iran locally

Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl; GAE, gallic acid equivalent; EO, essential oils.

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known as "Mohretalkh" (Javidnia et al., 2002). The essential oils of *S. mirzayanii* have been studied and the results have indicated that major components of the essential oil are linalyl acetate (11.8%), linalool (11.8%), α-terpinyl acetate (11%) and 1,8-cineole (8.7%) (Asadipour et al., 2004). The leaves of this plant have been used for many years in folk remedies both for gastritis relief and as an antiseptic agent (Javidnia et al., 2002). Some studies have reported on antimicrobial (Javidnia et al., 2009) and immunomodulatory activities of *S. mirzayanii* and also on its activities in cell-mediated and humoral antibody-mediated responses (Amirghofran et al., 2010). Although there are reports on the medicinal properties of *S. mirzayanii* (Javidnia et al., 2002), literature review has not revealed any previous research on the effects of abiotic stresses such as salinity on the volatile components, total phenol and antioxidants of this plant. The objective of this research was to identify the effects of salinity stress on the volatile components, total phenol and antioxidants of *S. mirzayanii*.

2. Materials and methods

2.1. Experimental

Experiments were carried out in the research greenhouse of the Biology Department, College of Sciences, Shiraz University, Shiraz, Iran. Experiments lasted for seven months from December 2011 to June 2012. Seeds of *S. mirzayanii* were kindly provided by the Research Center for Agriculture and Natural Resources, Shiraz, Iran. To break the seed dormancy, they were soaked in boiling water for 10 min and were then placed in Petri dishes moistened with distilled water and kept in a refrigerator (4 °) for 7 days. Seeds were then sown in plastic pots containing sands and powdered leaves (1:2) and were allowed to grow in the greenhouse with the mean day/night temperature and relative humidity of 29 ± 4 °C, 38 ± 5% and 17 ± 2 °C, 50 ± 5% respectively. Sixty days after seed germination, uniform seedlings with two nodes and four opposite leaves were transplanted into big plastic pots (30 × 50 cm). Each pot was filled with 10 kg of air-dried soil and two seedlings were used per pot for all treatments. Physical and chemical properties of the soil for plants are presented in Table 1. This was the same for all the plants. The only change was in the salt stress due to added salt in the water.

2.2. Irrigation

Eight weeks after transplanting, plants were subjected to different levels of salinity supplied with irrigation water. In order to prevent osmotic shock, salt solutions were added gradually at several stages and so, lasting for three weeks. To keep the levels of soil salt concentration constant, distilled water was used in subsequent irrigations. At the end of salt treatment, total soil electrical conductivities including control were determined by EC meter (0.40, 2.3, 4.5, 6.8 and 9.1 dS m^{-1}). Salt stress symptoms (leaf tip chlorosis and necrosis) in plants treated with high salt concentrations appeared after three weeks. At this time, seedlings were harvested. A total of 10 g of fresh leaf material was harvested per plant, 3.5 g of which was used for HGC–MS analysis and the rest was allowed to dry at room temperature.

2.3. Total phenolic extraction

Harvested leaves were dried at room temperature for one week. Leaves were extracted by stirring 200 mg of dry leaf powder in 2 ml methanol–acetic acid (85:15 v/v). The extracts were kept at −18 °C. After 24 h, extracts were sonicated for 15 min and centrifuged at 10,000 ×*g* in a refrigerated centrifuge for 20 min. The supernatant was further extracted by n-Hexane (1:1 v/v) and mixed thoroughly. The mixture was centrifuged at 10,000 ×*g* in a refrigerated centrifuge for 20 min. The total phenolic fraction (lower phase) was removed, filtered through a 0.2 μm membrane filter and stored at −18 °C for future analysis (Justesen et al., 1998).

2.4. Determination of total phenolics

Total phenolic content was determined by the Folin–Ciocalteu method (Singleton and Rossi, 1965). Two hundred microliters of 10-fold diluted [methanol–acetic acid (85:15 v/v)] samples of the extract was added to 1 ml of 1:10 diluted Folin–Ciocalteu reagent in test tubes. The mixture was shaken and allowed to stand for 6 min and then 800 μl of 7% Na_2CO_3 solution was added. After incubation for 90 min at 23 °C, 220 μl of each solution was added to 96 micro-plate wells and the solution absorbance was read at 760 nm by a micro-plate reader, model BioTek ELx 808. All samples were prepared in triplicate and read four times. A mixture of 7% Na_2CO_3 solution, 1:10 diluted Folin–Ciocalteu reagent and the extracting solution instead of plant extract was used as blank. Solutions of gallic acid (0–500 μg ml^{-1}) were used to prepare the standard curves (R^2 = 0.997). Results were expressed as milligram gallic acid equivalent (mg GAE)/g leaves dry weight.

2.5. Preparation of plant extracts for the analysis of antioxidant activities

Harvested leaves were dried at room temperature for 1 week. Leaf extracts were obtained by stirring 1 g of dry leaf powder with 10 ml pure methanol for 30 min. The extracts were then kept for 24 h at 4 °C, filtered through a Whatman No. 4 filter paper, the filtrate was dried using a rotary evaporator at 45 °C and stored at 4 °C for later analysis.

2.6. 1,1-Diphenyl-2-picrylhydrazyl (DPPH˚) scavenging activity

The effect of methanolic extracts on DPPH˚ degradation was estimated according to the Hanato et al. (1988) method. The powdery extracts (3.2 mg) were dissolved in 1 ml pure methanol making a final concentration of 3200 μg ml^{-1}. Each sample solution was diluted serially to final concentrations of 3.2, 6.25, 12.5, 25, 50, 100, 200, 400, 800, and 1600 μg ml^{-1}, in methanol. A total of 200 μl DPPH˚ methanolic solution (100 mmol l^{-1}) was added to 20 μl of each sample. The mixture was shaken vigorously and left standing at room temperature for 30 min. Absorbance of the solutions was then read at 517 nm after 30 min by a micro-plate reader model BioTek ELx 808. All samples were prepared in triplicate and their absorbance was read four times. The potential of extracts to scavenge DPPH˚ radicals was calculated using the following equation: DPPH radical scavenging activity (%) = 100 − [(Absorbance

Table 1
Physical and chemical properties of the soil.

Texture (%)			Cation and anion in saturated extract (m.e./l)										EC (dS m^{-1})	pH	TNV[a] (%)	OC[b] (%)
Sand	Silt	Clay	K^+	Na^+	Mg^{2+}	Ca^{2+}	Sum cation	SO_4^{-2}	Cl^-	HCO_3^-	Sum anion	P				
17.5	44.6	37.9	0.02	0.48	1	2.5	4	0.53	0.95	2.5	3.98	5.2	0.4	8.13	32	0.03

[a] TNV: Total Neutralizing Value.
[b] OC: Organic Carbon.

of sample − Absorbance of blank) × 100 / Absorbance of control]. Methanol (200 μl) plus extracting solution (20 μl) were used as blank, while DPPH solution plus methanol were used as negative control. The positive control was DPPH solution plus different concentrations of gallic acid. The free radical scavenging activities of the solutions were expressed as IC_{50} (mg ml^{-1}) which was calculated graphically using different concentrations of samples versus DPPH inhibition percentage. IC_{50} is the antiradical dose required to cause a 50% inhibition. Lower IC_{50} values correspond to higher antioxidant activities of the plant extract (Patro et al., 2005).

2.7. Headspace gas chromatography–mass spectrophotometric (HGC–MS) analysis

Fresh leaves of *S. mirzayanii* were crushed and 3.5 g of each sample was quickly placed in 20 ml headspace vials, and immediately was sealed with silicone rubber septa and aluminum caps. The vials were then transferred to the headspace tray. The headspace proceeded on the Combi-PAL system which was equipped with a headspace auto-sampler, heater and agitator. The vials were heated to 80 °C for 20 min while being agitated. The temperature of the sampling needle and transmission line was 85 °C.

2.8. Volatile oil composition

The leaf volatile components were analyzed using Agilent model 7890-A series gas chromatography and Agilent model 5975-C mass spectrometry. The HP-5 MS capillary column (phenyl methyl siloxane, 30 m × 0.25 mm i.d. × 25 μm) was used with helium gas at the rate of 1 ml/min as carrier. The GC oven temperature was programmed from 60 °C to 210 °C at the rate of 3 °C/min and then was increased from 210 °C to 240 °C at the rate of 20 °C/min and kept constant at 240 °C for 8.5 min. The split ratio was adjusted to 1:50 and the injection volume was 1000 μl. The injector temperature was 280 °C. The quadruple mass spectrometer was scanned over 40–550 amu with an ionizing voltage of 70 eV. Retention indices were determined using retention times of n-alkanes (C8–C25) that were injected after the essential oils under the same chromatographic conditions. The retention indices for all components were determined using n-alkanes as standard. The compounds were identified by comparing the retention indices (RI, HP-5) with those reported in the literature and also by comparing their mass spectra with the Wiley GC–MS Library, Adams Library, Mass Finder 2.1 Library data and published mass spectra data (McLafferty and Stauffer, 1989; Adams, 2007).

2.9. Statistical analysis

Three replications were used for each treatment. Data were expressed as means ± standard deviation (SD). The means were compared by using the one-way and multivariate analysis of variances (ANOVA) followed by Duncan's multiple range tests. The difference between individual means was deemed to be significant at $P < 0.05$.

3. Results

3.1. Effects of salinity on total phenolic content

The absorbance values of the extract solutions reacted with Folin–Ciocalteu reagent compared with the gallic acid standard solutions are shown in Table 2. Salt treatments increased the leaf total phenolic compounds significantly. At 6.8 dS m^{-1} NaCl, total phenol was 3.8 mg GAE g^{-1} DW which was 1.4 times higher than that of control leaves (2.7 mg GAE g^{-1} DW). However, increasing salinity level to 9.1 dS m^{-1} caused a significant reduction in leaf phenolic content (Table 2).

Table 2
Effect of salinity on phenolic content and antioxidant activity (IC_{50} values).

NaCl concentration (dS m^{-1})	Phenolic content (mg of GAE g^{-1} DW)	DPPH° scavenging activity IC_{50} (mg ml^{-1})
0.4 (control)	2.70 ± 0.02[b]	3.54 ± 0.4[c]
2.3	2.91 ± 0.01[c]	3.34 ± 0.1[bc]
4.5	3.24 ± 0.02[a]	3.00 ± 0.1[b]
6.8	3.82 ± 0.01[d]	2.13 ± 0.1[a]
9.1	3.22 ± 0.04[a]	2.19 ± 0.05[a]

Values (means of three replicates ± SD) of each parameter followed by at least one same letter are not significantly different at $P < 0.05$.

3.2. Effects of salinity on antioxidant activity

Antioxidant activity was determined by evaluating the effects of leaf extracts on DPPH free radical scavenging activities at all salt treatments. At 6.8 dS m^{-1} NaCl, leaf extracts with the IC_{50} value of 2.13 mg ml^{-1} displayed the highest DPPH free radical quenching activity as compared to other treatments (Table 2). These effects may be related to the higher total phenolic contents in *S. mirzayanii* leaf under 6.8 dS m^{-1} NaCl treatment. At 9.1 dS m^{-1} NaCl treatment, though the differences were not significant, the free radical scavenging activity of the leaf extracts decreased as compared to 6.8 dS m^{-1} NaCl treatments (Table 2).

3.3. Effects of salinity on volatile oil components

The amounts of volatile oil compounds in *S. mirzayanii* at different salt stresses are shown in Table 3. Twenty nine different compounds were identified in the leaves of control plants. The most of which were oxygenated monoterpenes (44.67%) comprising of α-terpinyl acetate (22.83%) and 1,8-cineole (18.61%), and sesquiterpene hydrocarbons (41.82%) represented mainly by bicyclogermacrene (17.89%).

Salinity stress at 2.3 dS m^{-1} decreased the amounts of sesquiterpene hydrocarbons and oxygenated sesquiterpenes by 16.43% and 25.29% respectively. It is to be noted that despite the decrease in sesquiterpenes, the amount of bicyclogermacrene remains as one of the major compounds (14.80%) among these volatile substances. On the other hand, under the same treatment, an increase of 27.83% of oxygenated monoterpenes was noted among which α-terpinyl acetate (32.10%) and 1,8-cineole (21.29%) were the highest. These increases were 40.60% and 14.40% higher respectively, as compared to the amounts found in control plants. Also, under the same treatment, a new compound, terpinen-4-ol was detected.

Increasing salt concentration to 4.5 dS m^{-1} NaCl enhanced the rates of both monoterpene hydrocarbons and oxygenated monoterpene biosynthesis. It is very interesting to note that among oxygenated monoterpenes, the biosynthesis of 1,8-cineole reached a maximum level (35.60%). Therefore, biosynthesis of 1,8-cineole, one of the main components of *S. mirzayanii* essential oils, increased by 91.29% at 4.5 dS m^{-1} NaCl salinity level as compared with control (Table 3).

At 6.8 dS m^{-1} NaCl treatment, the biosynthesis of a new compound, camphene, was induced. Also at this salt level, a 22.56% increase in sesquiterpene hydrocarbons was observed as compared to plants treated with 4.5 dS m^{-1} salinity. Although the amounts of the other group of terpenes decreased, the amounts of 1,8-cineole (35.42%) and α-terpinyl acetate (20.97%) remained at a constant high level.

In plants treated with 9.1 dS m^{-1} salinity, major changes in the percentages of volatile components occurred which resulted in the highest concentration of oxygenated monoterpenes (71.76%). Under this treatment, an increase in biosynthesis of linalyl acetate (19.85%) was observed as compared with other treatments including control. Therefore, at the salinity level of 9.1 dS m^{-1}, the main components of *S. mirzayanii* volatile components were shown to be: 1,8-cineole (26.76%), α-terpinyl acetate (22.48%), linalyl acetate (19.85%) and bicyclogermacrene (10.95%) (Table 3).

Table 3
HS-GC/MS analysis of volatile compounds in *Salvia mirzayanii* leaves under NaCl treatments (means of three replicates ± SD).

No.	Compounds (% FW)	KI*	NaCl concentrations (dS m^{-1})				
			0.4 (control)	2.3	4.5	6.8	9.1
1	α-Pinene	933	0.58 ± 0.07^{b}	0.72 ± 0.00^{a}	1.26 ± 0.06^{c}	0.97 ± 0.07^{d}	0.74 ± 0.06^{a}
2	Camphene	948	Nd	Nd	Nd	0.28 ± 0.04^{a}	Nd
3	Sabinene	972	0.5 ± 0.01^{c}	0.77 ± 0.07^{a}	0.90 ± 0.06^{d}	0.68 ± 0.07^{ab}	0.64 ± 0.07^{b}
4	β-Pinene	976	0.73 ± 0.14^{b}	1.20 ± 0.34^{a}	2.11 ± 0.15^{c}	1.61 ± 0.07^{d}	1.11 ± 0.07^{a}
5	Myrcene	989	0.49 ± 0.07^{a}	0.73 ± 0.07^{bc}	0.61 ± 0.06^{ab}	0.46 ± 0.00^{a}	0.86 ± 0.14^{c}
6	p-Cymene	1023	0.68 ± 0.12^{a}	0.43 ± 0.07^{b}	0.75 ± 0.15^{a}	1.10 ± 0.15^{a}	0.61 ± 0.06^{ab}
7	Limonene	1027	1.26 ± 0.05^{a}	1.27 ± 0.00^{a}	1.26 ± 0.05^{a}	1.15 ± 0.01^{b}	1.03 ± 0.00^{c}
8	**1,8-Cineole**	**1029**	**18.61 ± 0.14^{b}**	**21.29 ± 0.05^{c}**	**35.6 ± 0.36^{a}**	**35.42 ± 0.15^{a}**	**26.76 ± 0.07^{d}**
9	γ-Terpinene	1056	0.22 ± 0.00^{c}	0.26 ± 0.03^{a}	0.31 ± 0.00^{b}	0.44 ± 0.00^{d}	0.29 ± 0.04^{ab}
10	Linalool	1098	0.29 ± 0.07^{a}	0.16 ± 0.07^{ab}	0.09 ± 0.01^{b}	0.57 ± 0.15^{c}	0.75 ± 0.07^{d}
11	δ-Terpineol	1164	0.24 ± 0.00^{a}	0.40 ± 0.06^{b}	0.22 ± 0.07^{a}	0.58 ± 0.14^{c}	0.43 ± 0.04^{b}
12	Terpinene-4-ol	1175	Nd	0.10 ± 0.00^{a}	0.103 ± 0.02^{a}	0.40 ± 0.08^{b}	0.14 ± 0.07^{a}
13	α-Terpineol	1188	1.34 ± 0.15^{a}	2.14 ± 0.21^{c}	0.46 ± 0.06^{b}	1.26 ± 0.00^{a}	0.58 ± 0.00^{b}
14	**Linalyl acetate**	**1253**	**1.14 ± 0.00^{a}**	**0.63 ± 0.05^{b}**	**0.62 ± 0.02^{b}**	**1.22 ± 0.06^{a}**	**19.85 ± 0.15^{c}**
15	δ-Elemene	1334	2.29 ± 0.21^{a}	2.13 ± 0.08^{a}	0.90 ± 0.07^{b}	1.22 ± 0.07^{a}	0.86 ± 0.06^{b}
16	**α-Terpinyl acetate**	**1347**	**22.83 ± 0.25^{a}**	**32.10 ± 0.21^{b}**	**26.75 ± 0.14^{c}**	**20.97 ± 0.07^{d}**	**22.48 ± 0.08^{a}**
17	β-Elemene	1389	4.32 ± 0.35^{b}	3.62 ± 0.04^{c}	2.40 ± 0.04^{a}	2.65 ± 0.01^{a}	1.89 ± 0.00^{d}
18	β-Longipinene	1400	0.40 ± 0.06^{a}	0.13 ± 0.00^{b}	Nd	0.30 ± 0.07^{c}	Nd
19	Longifolene	1406	3.61 ± 0.05^{c}	2.69 ± 0.07^{a}	2.67 ± 0.15^{ab}	3.03 ± 0.00^{d}	2.49 ± 0.17^{b}
20	α-Gurjunene	1416	4.64 ± 0.07^{a}	3.25 ± 0.00^{b}	2.54 ± 0.06^{c}	3.10 ± 0.07^{d}	1.62 ± 0.08^{e}
21	β-Gurjunene	1437	3.52 ± 0.09^{b}	1.23 ± 0.00^{a}	1.32 ± 0.14^{a}	4.10 ± 0.05^{c}	0.99 ± 0.00^{d}
22	Allo-aromadendrene	1456	2.14 ± 0.00^{a}	0.82 ± 0.05^{b}	0.51 ± 0.09^{c}	1.29 ± 0.11^{d}	0.07 ± 0.02^{e}
23	γ-Gurjunene	1473	0.34 ± 0.04^{a}	0.25 ± 0.00^{b}	0.18 ± 0.01^{c}	Nd	0.07 ± 0.00^{d}
24	β-Selinene	1483	0.18 ± 0.02^{a}	0.103 ± 0.00^{b}	0.14 ± 0.02^{c}	Nd	Nd
25	Bicyclogermacrene	1493	17.89 ± 0.32^{b}	14.80 ± 0.20^{c}	11.83 ± 0.09^{a}	12.05 ± 0.07^{a}	10.95 ± 0.07^{d}
26	Cis-dihydroagarofuran	1516	1.14 ± 0.19^{b}	0.88 ± 0.13^{c}	0.28 ± 0.00^{a}	0.31 ± 0.02^{a}	0.20 ± 0.02^{a}
27	δ-Cadinene	1520	0.39 ± 0.07^{a}	0.53 ± 0.00^{b}	0.35 ± 0.05^{a}	0.60 ± 0.00^{b}	0.35 ± 0.04^{a}
28	Unknown	1522	0.70 ± 0.04^{b}	0.57 ± 0.02^{c}	0.16 ± 0.03^{a}	0.15 ± 0.03^{a}	0.09 ± 0.00^{d}
39	Germacrene D-4-ol	1571	1.44 ± 0.00^{a}	1.34 ± 0.00^{b}	0.72 ± 0.06^{c}	0.44 ± 0.00^{d}	0.55 ± 0.02^{e}
30	5-Neo-cedranol	1686	7.40 ± 0.06^{b}	5.50 ± 0.06^{c}	4.92 ± 0.04d	3.65 ± 0.06^{a}	3.58 ± 0.07^{a}
Total identified classes							
Monoterpene hydrocarbons			4.24 ± 0.42^{b}	5.12 ± 0.33^{a}	6.93 ± 0.28^{c}	6.25 ± 0.29^{d}	4.99 ± 0.18^{a}
Oxygenated monoterpene			44.67 ± 0.34^{a}	57.98 ± 0.35^{b}	64.37 ± 0.62^{c}	60.87 ± 0.63^{d}	71.28 ± 0.35^{e}
Sesquiterpene hydrocarbons			41.82 ± 0.48^{a}	34.95 ± 0.02^{b}	23.62 ± 0.25^{c}	28.95 ± 0.26^{d}	19.83 ± 0.15^{e}
Oxygenated sesquiterpene			8.54 ± 0.26^{b}	6.38 ± 0.17^{c}	5.20 ± 0.06^{d}	3.96 ± 0.05^{a}	3.78 ± 0.07^{a}

KI*: Confirmed by comparison with Kovats indices on DB5 column (Adams, 2007).
Nd: not detected.
Values followed by the same small letter did not share significant differences at 5% (Duncan's test).
The bold figures represent the highest amount of pharmaceutical substances found in *S. mirzayanii*.

4. Discussion

In higher plants, the surplus carbon not used for growth can be allocated to carbon-based secondary compounds (CBSC) or to storage compounds such as starch. Carbon allocated to CBSC is shunted into several alternative pathways (shikimic acid or mevalonic acid pathways), leading to the synthesis of major classes of CBSC such as phenylpropanoids, hydrolysable tannins and terpenoids (Koricheva et al., 1998). The levels of plant CBSC and proportional allocation of available carbon to individual CBSC such as phenolics and terpenoids are partly under genetic control (Berenbaum and Zangerl, 1992) and are determined in part by environmental conditions (Waterman and Mole, 1989). Intraspecific variation in CBSC concentrations is therefore an important factor that needs to be considered when evaluating plant resistance to stresses.

In the present study, both phenolic contents and antioxidant activities of *S. mirzayanii* leaves were increased under mild (6.8 dS m^{-1}) salinity stress (Table 2). However, high salinity (9.1 dS m^{-1}) reduced both of these two parameters. The increase in phenolic contents in different plant tissues under increasing salinity has also been reported in a number of plants (Muthukumarasamy et al., 2000). Navarro et al. (2006) reported an increase in total phenolic content in red pepper plants under moderate salinity levels.

Plants vary widely both in their phenolic compositions and contents which are controlled both genetically and environmentally (Awika and Rooney, 2004). Phenolic compounds exhibit antioxidant activity by inactivating lipid free radicals or by preventing the decomposition of hydroperoxides into free radicals (Pokorny et al., 2001). The degree of cellular oxidative damage in plants exposed to abiotic stress is controlled by the capacity of the plants to produce antioxidant agents. Therefore, salt tolerance seems to be favored by the increase in plant antioxidant levels to detoxify the reactive oxygen species produced under these conditions (Noctor and Foyer, 1998). In our study, the tolerance of *S. mirzayanii* to moderate salinity (2.3–6.8 dS m^{-1}) treatments coincided with the leaf enrichment in total phenols while their tolerance to high salinity (9.1 dS m^{-1}) paralleled with a decrease in the amount of these compounds. These results show concomitant stimulations in both phenolic biosynthesis and antioxidant activity in *S. mirzayanii* tissues when exposed to moderate NaCl salinity, findings that support our hypothesis that plants exhibiting tolerance to salt stress are useful systems for the production of secondary metabolites serving both as food stuff and medicinal products.

There are some reports on the composition of *S. mirzayanii* (wild type) essential oils. According to these reports the major components of *S. mirzayanii* essential oils are linalyl acetate (11.8%), linalool (11.8%), α-terpinyl acetate (11%) and 1,8-cineole (8.7%) (Asadipour et al., 2004; Yamini et al., 2007). However, there is no information about the NaCl effects on these compositions. In the present study, the main changes caused by NaCl treatments were related to the relative proportion of these secondary metabolites, synthesis of new ones and disappearance of others (Table 3). Moderate NaCl salinity was shown to improve the yields of volatile compounds in *S. mirzayanii*. Thus, *S. mirzayanii* plants cultivated under NaCl salinity may provide a potential source of volatile substances. Our results also showed that the leaves of *S. mirzayanii* are rich in α-terpinyl acetate, 1,8-cineole

and bicyclogermacrene. Salt treatment enhanced the production of α-terpinyl acetate at 2.3 dS m^{-1}, 1,8-cineole at 4.5 dS m^{-1} and linalyl acetate at 9.1 dS m^{-1} at the highest levels. These compounds are of special interest to pharmacologists and pharmaceutical companies. The compound 1,8-cineole that was particularly produced in high amounts at 4.5 dS m^{-1} NaCl salinity has shown antimicrobial effects against many bacteria, including *Mycobacterium tuberculosis* and methicillin-resistant *Staphylococcus aureus* (MRSA), viruses and fungi (including *Candida*). Surprisingly, antimicrobial substances also have immunostimulatory, anti-inflammatory, antioxidant analgesic and spasmolytic effects (Sadlon and Lamson, 2010). The Cardamom (*Elettaria cardamomum* L.) seed extract which is characterized by having high (36.8%) terpinyl acetate contents, demonstrates antimicrobial activity against some of the most widely distributed pathogenic and infesting bacteria in foods (Guchev et al., 2012). Linalool and linalyl acetate play a major role as anti-inflammatory agents (Peana et al., 2002). There are contradictory reports in the literature concerning changes in essential oil quality in response to salt stress. For example, there are reports on the increase in essential oils and on their composition in response to low levels of salinity in *Satureja hortensis* (Baher et al., 2002), in sage (Hendawy and Khalid, 2005) and in thyme (Ezz El-Din et al., 2009). In contrast, other reports have shown a significant reduction in essential oils in lemon balm and in sweet marjoram (*Majorana hortensis* L.) (Shalan et al., 2006). Also, some reports indicate that the compositions of essential oils are modified in moderate and high salinity. In *Salvia officinalis* in control and plants treated with 25 mM NaCl, the main essential oil has been viridiflorol. However, at higher levels of salinity (50 and 75 mM NaCl), 1,8-cineole and at 100 mM NaCl, manool have been the predominant essential oils (Ben Taarit et al., 2010).

These variations could be due to the induction of specific enzymes involved in the biosynthesis of essential oil compounds at each level of salt stress (Burbott and Loomis, 1969). As a whole, the parallel stimulations in volatile oil composition, antioxidant activities and total phenolic content in *S. mirzayanii* leaf tissues at moderate salinity, support our assumption that plants tolerant to salt stress are potentially useful systems for the production of secondary metabolites to be used by both food and pharmaceutical industries.

5. Conclusion

Various industries are now looking for sources of alternative, more natural and environmentally friendly antimicrobials, antibiotics, antioxidants and crop protection agents. The biological activity of the essential oils can be compared with the activity of synthetically produced pharmaceutical products. Our study showed that moderate salinity could induce *S. mirzayanii* to produce high amounts of some valuable volatile oils and total phenolic compounds. Therefore, the development of new chemotypes at different salt levels could be considered as valuable aspects of salinity stress in some plants inducing them to produce compounds with industrial and pharmaceutical interest.

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